polyclonal antibody (CY-048, Innogenetics, Belgium) as a capture antibody, biotinylated mouse anti-rat monoclonal antibody (CY-106 clone BD-1, Innogenetics) as a detection antibody, and Alkaline phosphatase-Streptavidin (cat No. 43-4322, Zymed, SF, CA) with rat recombinant IFN-γ as a standard (Cat No 3281SA, Gibco BRL); TNF-α, commercial semi-ELISA kit for the detection of rat TNF-α, (Cat No 80-3807-00, Genzyme, Cambridge, MA); IL-4, mouse anti-rat IL-4 monoclonal antibody (24050D OX-81, PharMingen, San Diego, CA) as a capture antibody, and rabbit anti-rat IL-4 biotin-conjugated polyclonal antibody (2411-2D, PharMingen) as second antibody. Recombinant rat IL-4 purchased from R&D (504-RL) was used as a standard.

Statistical analysis: Significance of differences was examined using Student's t-test. Mann-Whitney sum of ranks test was used to evaluate significance of differences in mean of maximal clinical score (Figure 3). Value of $p < 0.05$ was considered significant.

Experimental Results

IGIF mRNA is transcribed in the inflamed EAE brain: Midbrain-brain stem samples were obtained from rats with developing transferred EAE (Figure 1A) before adoptive transfer of disease (day 0), and at various time points: before the onset of disease (day 3), at the day of onset (day 5), the peak (day 7), following recovery (day 10), and 10 days after recovery (day 20). For each time point, samples from six different brains were subjected to RT-PCR analysis using specific oligonucleotide primers which constructed to IGIF and IFN-γ . Each amplification was calibrated to β-actin and verified by Southern blot analysis. This enabled semi-quantitative analysis of the dynamics of mRNA transcription of IGIF and IFN-γ at the site of inflammation. Figures 1C and 1E show representative results from each time point of the experiment. A substantial increase in the transcription of both IGIF and IFN-γ mRNA in EAE brains was observed at the peak of disease (day 7). The augmented transcription of IFN-γ mRNA reverted to background levels during recovery. Unexpectedly, a notable transcription of IGIF mRNA could be observed even ten days after recovery (Figure 1C).

Rats with developing active disease manifested similar mRNA transcription characteristics as those with developing transferred disease. That is, a substantial increase in the transcription of both IGIF and IFN-γ mRNA in EAE brains was observed at the peak of disease (day 13). The

augmented transcription of IFN-γ, but not of IGIF mRNA, regressed to background level during recovery (Figures 1D and 1F). A substantial increase in the level of a IGIF transcription at the site of inflammation in the CNS during the course of disease may suggest involvement in its regulation. To evaluate this point, the role of IGIF in regulation of EAE was investigated.

Recombinant rat IGIF and its neutralizing antibodies affect IFN-γ production by activated T cells from naive donors more significantly than by antigen specific primed T cells: PCR products encoding rat IGIF were used to generate the recombinant protein which was used to produce anti-IGIF neutralizing antibodies. These antibodies significantly reduced the production of IFN-γ in primed T cells proliferating in response to their specific myelin basic protein (MBP) epitope (Figure 2C 3.2 ± 0.25 versus 1.8 ± 0.11 ng/ml with backgrounds of 0.2 ± 0.1 and 0.25 ± 0.1, $p < 0.01$) and entirely blocked IFN-γ production in Con A activated T cells from naive donors (Figure 2A, 5.1 ± 0.4 versus 0.4 2 ± 0.1 ng/ml with backgrounds of 0.4 ± 0.1 and 0.36, $p < 0.001$). Control IgG from normal rabbit serum did not exert a notable effect on IFN-γ production by either Con A activated naive spleen cells or MBP p68-86 primed spleen cells (data not shown). Recombinant rat IGIF elicited IFN-γ production in Con A activated splenic T cells from naive donors (Figure 2B, 15.8 ± 0.8 ng/ml versus 5.1 ± 0.3 with backgrounds of 0.3 ± 0.1 and 0.4 ± 0.15, $p < 0.001$) and significantly, though again less profoundly, the response of primed spleen T cells to their target MBP antigen (Figure 2D, 4.97 ± 0.15 ng/ml versus 3.2 ± 0.25, with backgrounds of 0.3 ± 0.15 and 0.25 ± 0.1, $p < 0.001$). Thus, both the inhibitory effect of IGIF neutralizing antibodies and the augmentation by IGIF of IFN-γ production are more profound on activated T cells from a naive donor than on primed T cells responding to their target epitope. It has recently been suggested that IGIF primarily affects IFN-γ production by Th1 not Th2 cells (29). It is possible that immunization with p68-86/CFA induces a substantial selection of antigen specific Th2 cells, albeit not enough to inhibit the subsequent development of a Th1 mediated autoimmune disease.

The *in vitro* addition of either anti-IGIF antibodies or of recombinant IGIF did not affect the antigen specific proliferative response developed in primed splenic T cells responding to MBP p68-86 (SI = 4.2 ± 0.3, 3.6 ± 0.4 and 3.9 ± 0.3 in control spleen T cells) versus cultured spleen cells supplemented with either anti-IGIF antibodies or recombinant IGIF.

Neutralizing antibodies to recombinant rat IGIF block the development of both active and transferred EAE: The role of anti-IGIF antibodies in the regulation of T cell mediated autoimmune diseases has never been explored before. Herein the competence of the anti-IGIF neutralizing antibodies to inhibit active (Figure 3A) and transferred (Figure 3B) EAE is evaluated. Lewis rats were immunized with p68-86/CFA to develop active EAE. Just before the onset of disease (days eight and ten) and at the onset of disease (day eleven) these rats were injected with either rabbit anti-rat IGIF (IgG fraction), IgG fraction purified from non-immunized rabbits (control IgG), or with PBS, and monitored for clinical signs of EAE. Control PBS treated rats and rats treated with control IgG all (6/6 rats in each group) developed severe EAE (Mean maximal clinical score 3.3 ± 0.43 and 2.66 ± 0.26, respectively). In contrast, rats treated with anti-IGIF antibodies developed a markedly reduced disease (Figure 3A, incidence 5/6, mean maximal clinical score 1.2 ± 0.2, $p < 0.01$).

The competence of anti-IGIF antibodies to inhibit transferred EAE (Figure 3B) was further evaluated. Three, five and seven days after adoptive transfer of disease rats were injected as described above and monitored for clinical signs of EAE. While control PBS treated rats and rats treated with control IgG have all (6/6 rats in each group) developed EAE (mean maximal clinical score 1±0 in each group) rats administered with anti-IGIF antibodies were highly protected (Figure 3B, incidence 1/6, mean maximal clinical score 0.2 ± 0.1, $p < 0.01$). Thus, immunotherapy with anti-IGIF serves as a powerful tool to block the development of actively induced or transferred EAE.

Alteration of IFN-γ and IL-4 production in EAE rats injected with anti-IGIF neutralizing antibodies suggests that perturbation of the Th2/Th1 balance contributes to disease blockade: The possible involvement of a Th2/Th1 switch in EAE inhibition by anti-IGIF immunotherapy has been evaluated (Figure 4). Lewis rats were immunized with p68-86/CFA to develop active EAE. Five and seven days later these rats were injected with either PBS, control rabbit IgG or rabbit anti-rat IGIF (IgG fraction). Two days after the last treatment, splenic T cells were cultured with MBP p68-86 in stimulation medium that was (Figures 4C and 4D) or was not supplemented with recombinant rat IL-4 (Figures 4A and 4B). In spleen cells cultured from MBP 68-86 primed donors, IFN-γ was produced only when the priming antigen was added to the culture (Figures 4A, 0.3 ± 0.1 ng/ml without addition of MBP 68-86 versus 13.5 ± 0.7 in